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Brain Metabolic Changes Across King's Stages in Amyotrophic Lateral Sclerosis: a 18F-2-fluoro-2-deoxy-D-glucose-Positron Emission Tomography Study

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Brain Metabolic Changes Across King's Stages in Amyotrophic Lateral Sclerosis: a ^{18}F-2-fluoro-2-deoxy-D-glucose-Positron Emission Tomography Study

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Antonio Canosa had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors have approved the submitted version of the article. The information reported in the manuscript has never been reported elsewhere.

Abstract

Purpose. To assess the brain metabolic correlates of different regional extent of ALS, evaluated with the King's staging system, using brain ^{18}F-2-fluoro-2-deoxy-D-glucose-PET (^{18}F-FDG-PET)

Methods. 390 ALS cases with King's stage 1, 2, and 3 (n=390), i.e. involvement of 1, 2, and 3 body regions respectively, underwent brain ^{18}F-FDG-PET at diagnosis. King's stage at PET was derived from ALSFRS-R and was regressed out against whole brain metabolism in the whole sample. The full factorial design confirmed the hypothesis that differences among groups (King's 1, King's 2, King's 3, and 40 healthy controls - HC) existed overall. Comparisons among stages and between each group and HC were performed. We included age at PET and sex as covariates.

Results. Brain metabolism was inversely correlated with stage in medial frontal gyrus bilaterally, and right precentral and postcentral gyri. The full factorial design resulted in a significant main effect of groups. There was no significant difference between stages 1 and 2. Comparing stage 3 to stage 1+2, a significant relative hypometabolism was highlighted in the former in the left precentral and medial frontal gyri, and in the right medial frontal, postcentral, precentral, and middle frontal gyri. The comparisons between each group and HC showed the extension of frontal metabolic changes from stage 1 to stage 3, with the larger metabolic gap between stage 2 and 3.

Conclusions. Our findings support the hypothesis that in ALS the propagation of neurodegeneration follows a corticofugal, regional ordered pattern, extending from the motor cortex to posterior and anterior regions.

Keywords: Amyotrophic Lateral Sclerosis, ^{18}F-FDG-PET, King's staging system

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons, leading to muscle weakness and wasting that progressively spreads across body regions. In about 50% of cases also prefrontal regions are involved, causing various degrees of cognitive impairment of the frontotemporal type [1]. Death usually occurs within 2-5 years due to respiratory failure [2]. It has been proposed that ALS pathology disseminates in a regional ordered sequence, following a cortico-efferent spreading model [3].

In recent years the King's staging system has been proposed for ALS, mainly based on the extension of the disease across body regions [4]. It defines the following stages: 1, symptom onset (involvement of the first region); 2A, diagnosis; 2B, involvement of a second region; 3, involvement of a third region; 4A, need for gastrostomy; 4B, need for respiratory support (non-invasive ventilation). Central nervous system region involvement was defined by the presence of weakness, wasting, spasticity, dysphagia or dysarthria. Regions were defined in the same way as for the El Escorial criteria [5]. Afterwards, an algorithm based on ALSFRS-R has been proposed to estimate the King's stages [6].

Published data about the neuroimaging correlates of King's stages are limited to few Magnetic Resonance Imaging (MRI) studies based on small samples [7–9]. The aim of this study was to assess the brain metabolic correlates of different regional extent of ALS, evaluated according to the King's staging system, using brain ^{18}F-2-fluoro-2-deoxy-D-glucose-PET (^{18}F-FDG-PET), since it is a measure of neuronal injury and degeneration *in vivo* [10].

Materials and Methods

Study partecipants

Patients diagnosed with definite, probable and probable laboratory-supported ALS according to El Escorial revised diagnostic criteria [5], who underwent brain ^{18}F-FDG-PET at diagnosis between 2008 and 2015 at the ALS Centre of Turin ("Rita Levi Montalcini" Department of Neuroscience, University of Turin, Turin, Italy) were considered eligible for the study (n=406). The present study includes patients whose brain ^{18}F-FDG-PET scan was included in the analyses performed in previous publications.

Forty healthy controls (HC) were included. Subjects who were referred to the PET Centre for suspected lung cancer but in whom no oncologic disease was detected with ^{18}F-FDG-PET/CT and who had a normal neurological assessment were considered eligible as controls. Otherwise, major systemic illnesses, major vision disturbances, psychiatric illnesses, and diseases affecting brain functioning and metabolism represented exclusion criteria.

Genetic Analysis

All patients underwent genetic analysis for *C9ORF72*, *SOD1*, *TARDBP*, and *FUS* genes. All the coding exons and 50 bp of the flanking intron-exon boundaries of *SOD1*, of exon 6 of *TARDBP*, and of exons 14 and 15 of *FUS* have been PCR amplified, sequenced using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems Inc.), and run on an ABIPrism 3500 genetic analyzer. These exons were selected as the vast majority of known pathogenic variants are known to lie within these mutational hotspots. A repeat-primed PCR assay was used to screen for the presence of the GGGGCC hexanucleotide expansion in the first intron of *C9ORF72*.

^{18}F-FDG-PET acquisition

^{18}F-FDG-PET was performed according to published guidelines [11]. Patients fasted at least six hours before the exam. Blood glucose was <7.2 mmol/l in all cases before the procedure. After a 20-minute rest, about 185 MBq of ^{18}F-FDG was injected. The acquisition started 60 minutes after the injection. In the patient group a whole-body scan was performed setting head-first. In the control group a separate brain scan was performed after the whole-body one with a time difference of 15 minutes. The^{18}F-FDG-PET acquisition procedure was performed in the same environmental conditions in patients and controls, according to published guidelines [11]. PET/CT scans were performed on a Discovery ST-E System (General Electric). Brain CT (slice thickness of 3.75 millimetres, 140 kV, 60-80 mAs) and PET scan were sequentially acquired, the former being used for attenuation correction of PET data. The PET images were reconstructed with 4 iterations and 28 subsets with an initial voxel size of 2.34 x 2.34 x 2.00 mm and data were collected in 128×128 matrices.

Assessment of King's stage

The King's staging is based on the spreading of motor symptoms in three different body regions (bulbar, upper limbs, and lower limbs), and on the use of non-invasive ventilation (NIV) and enteral nutrition. We used the algorithm proposed by Balendra et al. [6] to calculate King's stage from ALSFRS-R. The bulbar region was considered involved if a patient lost any points on any of the three items regarding speech, salivation or swallowing (items 1, 2, and 3). The upper limb region was considered involved if a patient lost any points on either of the two items regarding handwriting and ability to cut food and handle utensils (items 4 and 5A). The lower limb region was considered involved if a patient lost any points on the item regarding walking (item 8). The presence of

gastrostomy was confirmed by the assessment of item 5B (evaluation of the ability to manipulate fastenings if a patient has a gastrostomy) rather than item 5A (answered by patients without gastrostomy). If a subject scored 0 points on question 10 (indicating that the patient has significant difficulty with dyspnoea and is considering using mechanical respiratory support) or less than 4 points on question 12 (dropping any points on this question indicates that Bi-level Airway Pressure ventilation is being used), this indicated that the patient was using NIV. We classified patients according to the following five stages of the King's staging system: 1, one region involved; 2, two regions involved; 3, three regions involved; 4A, patient needs gastrostomy; 4B, patient needs NIV. Since our aim was to evaluate the metabolic changes related to different regional extent of ALS, we focused on patients classified as King's stages 1, 2 and 3 (n=390). Indeed, stages 1, 2, and 3 correspond to involvement of 1, 2, and 3 body regions respectively, whereas stages 4A and 4B correspond to the reaching of milestones related to functional impairment (i.e. gastrostomy and NIV).

Statistical Analysis

The demographic and clinical characteristics of patient groups (King's Stage 1, 2, and 3) and HC were compared as follows. The χ^2-test was employed for categorical variables. The Analysis of Variance (ANOVA) or the Kruskal-Wallis test were used for quantitative, continuous variables. The homogeneity assumption needed for ANOVA was evaluated through the Levene's test. In the case of a significant Levene's test, the Kruskal-Wallis test was employed instead of ANOVA.

SPM12 implemented in Matlab R2018b (MathWorks, Natick, MA, USA) was used for image spatial normalization to a customized brain ^{18}F-FDG-PET template [12]. Intensity normalization was performed using the 0.8 default SPM value of grey matter threshold and images were subsequently smoothed with a 10-mm filter and submitted to statistical analysis.

In our sample (n =390) King's stage (1, 2, 3) was regressed out against whole brain metabolism. The SPM12 Multiple Regression routine was implemented with age at PET and sex as covariates and the height threshold was set at P<0.001 (P<0.05 FWE-corrected at cluster level). We used the full factorial design as implemented in SPM12 to test the hypothesis that differences among groups (King's 1, King's 2, King's 3, HC) exist overall (i.e., main effect of groups). In case the hypothesis was confirmed, comparisons between groups defined according to King's stage were performed through the two-sample t-test model of SPM12. For both analyses age at PET and sex were used as covariates and the height threshold was set at P<0.001 (P<0.05 FWE-corrected at cluster level). Although the scope of the study was the assessment of metabolic differences across King's stages, for a more exhaustive characterization of patient metabolic state, each group defined according to King's stage was compared to HC, through the two-sample t-test model of SPM12, with age at PET and sex as covariates, setting the height threshold at P<0.0001 (P<0.05 FWE-corrected at cluster level). In all the analyses only clusters containing >125 contiguous voxels were considered significant. Brodmann areas (BAs) were identified at a 0–2-mm range from the Talairach coordinates of the SPM output isocentres corrected by Talairach Client (http://www.talairach.org/index.html).

Results

Demographic and clinical data

Based on the ALSFRS-R at the time of PET, 165 patients (42.3%) were in King's stage 1, 133 (34.1%) in stage 2, and 92 (23.6%) in stage 3. Demographic and clinical characteristics of patients belonging to the three groups and of the whole sample are reported in Table 1. We found a significant difference among the three groups for site of onset (p<0.001), ALSFRS-R total score (p<0.001), and cognitive status as defined by diagnostic criteria published by Strong et al. [13]

(p=0.019). However, a bivariate correlation analysis did not show any correlation between site of onset and King's stage (p=0.36). Regarding the difference in ALSFRS-R total score, this could be expected since the King's Stage has been calculated based on the ALSFRS-R score according to the algorithm published by Balendra et al. [6] As for the differences in cognitive status, these confirm a previous study showing that cognitive impairment in ALS tends to be more severe as the King's clinical stage increases [14].

In the HC group the median age was 66.5 years (Interquartile Range 55.0-72.0), and the male/female ratio was 2.64 (29/11). In the comparisons between each stage group and HC we found no significant difference for age and sex distribution, with the exception of sex distribution between King's stage 3 and HC (p=0.002). Nevertheless, both age and sex were included as covariates in the analyses.

^{18}F-FDG-PET data

Using multiple regression analysis we found an inverse correlation between brain metabolism and King's stage (with stage 3 on the relatively hypometabolic side and stage 1 on the relatively hypermetabolic side). We identified clusters including bilateral medial frontal gyrus (Brodmann Area, BA, 6), right precentral gyrus (BA 4), and right postcentral gyrus (BA 2) (Figure 1, Table 2). We did not identify any cluster with positive correlation.

The full factorial design resulted in a significant main effect of groups (Supplemental Figure 1). We hence computed the *post-hoc* comparisons between the four groups. Comparing King's stage 1 and King's stage 2 groups, we did not find any significant difference. Therefore, we merged such groups into a King's 1+2 group. The comparison between the King's 1+2 group and the King's 3 group revealed a significant relative hypometabolism in the King's 3 group in clusters including left precentral and medial frontal cortex (BAs 4 and 6), and right medial frontal, postcentral, precentral

and middle frontal cortex (BAs 4, 6, 3, 9, and 8) (Figure 2, Table 3). The King's 3 group did not show any cluster of relative hypermetabolism as compared to the King's 1+2 group.

In the comparison with HC we identified two clusters of relative hypometabolism in patient groups. The former included occipito-temporo-parietal regions and did not show a clear pattern of extension along the increase of clinical stage. The latter included frontal regions and showed an extension from left to right emisphere from King's stage 1 to stage 2, and a marked bilateral extension in stage 3 (Supplemental Figures 2, 3, and 4). No clusters of relative hypermetabolism in patient groups as compared to HC were found.

Discussion

In this study we explored the ^{18}F-FDG-PET correlates of ALS clinical stages according to King's staging system. We focused on King's stage 1, 2 and 3, corresponding to the spreading of motor impairment to 1, 2, and 3 body regions respectively. A full factorial analysis showed that group differences exist at global level and overlap the ones found between groups and groups and controls.

In the multiple regression analysis brain metabolism negatively correlated with King's stage. We found a decreasing gradient of metabolism going from King's stage 1 to King's stage 3 in clusters including right precentral and postcentral gyrus (BAs 4 and 2), and bilateral medial frontal gyrus (BA 6). When performing group comparisons, no significant difference was detected between King's stage 1 group and King's stage 2 group. King's stage 3 group showed relatively hypometabolic clusters when compared to King's stage 1+2 group, including left precentral and medial frontal gyrus (BAs 4 and 6) and right precentral, middle frontal, postcentral, and medial frontal gyrus (BAs 4, 6, 3, 8, and 9). The comparisons between each stage group and HC resulted in

agreement with such findings, showing the extension of frontal metabolic changes from stage 1 to stage 3, with the larger metabolic gap between stage 2 and 3. Posterior clusters, including occipital lobes in all comparisons, of relative hypometabolism in ALS patients as compared to HC have already been reported [15].

The corticospinal tracts (CST) originate from neurons mainly situated in BA 4, corresponding to the primary motor cortex. Nevertheless, CST fibers rise also from neurons situated in other cortical regions, including premotor and supplementary motor cortex (BA 6), and primary somatosensory cortex (BAs 1, 2, and 3). Therefore, we identified a decreasing gradient of metabolism going from patients with King's stage 1 to subjects with King's stage 3 in cortical regions from which the corticospinal tracts originate. Furthermore, group comparisons suggested that the main metabolic gap is situated between stage 2 and 3. We detected no difference between subjects with King's stage 1 and 2. A possible explanation is that patients showing the involvement of three body regions at diagnosis subtend a more rapid neurodegenerative process, involving prefrontal cortex to a greater extent, as compared to patients with stage 1 and 2. Otherwise, the metabolic difference between stage 1 and 2 is probably under threshold, being the neurodegenerative process slower and less extensive.

Clusters of relative hypometabolism in subjects with King's stage 3 as compared to patients with stage 1 and 2 included BAs 4, 6 and 3 and extended towards anterior regions (BAs 8 and 9). In a previous study we identified a decreasing gradient of metabolism going from ALS with normal cognition to ALS with FTD, through cases with intermediate cognitive deficits (ALS-Ci) in frontal clusters including BAs 8 and 9 [16]. Synapse degeneration in BA 9 has been reported as a strong predictor of cognitive impairment in ALS [17]. Our findings are in agreement with neuropathological data suggesting that phosphorylated TDP-43 (p-TDP-43) tends to spread from motor cortex to prefrontal and postcentral regions via axonal pathways [3]. According to such

staging of p-TDP-43 pathology in ALS, BAs 4 and 6 are involved in stage 1, while the extension to prefrontal and postcentral cortex is reported in stage 3. The data of the present study seem to strengthen the findings of our previous population-based cross-sectional study on cognitive impairment across ALS clinical stages [14], suggesting that cognitive deficits tend to be more severe as the clinical stage increases. In the proposed staging of pTDP-43 pathology [3] the neurodegenerative process was thought to spread along axonal pathways. Our results are in agreement with such hypothesis, that is further strengthened by several MRI studies, aimed at tracking the spreading of the neurodegenerative process *in vivo* through diffusion tensor imaging (DTI) [18–20], and simulation models based on brain network analyses [21,22].

A ^{18}F-FDG-PET study [23] on 146 patients evaluated the possible correspondence between the key brain regions used in the assessment of the Brettschneider's neuropathological staging system [3], and metabolic patterns allowing *in vivo* staging of ALS. This study found that the *post mortem* neuropathological stages corresponded to distinct metabolic patterns.

The cross-sectional design of our study may have limited our findings. Nevertheless, patients were tested early after diagnosis and the cognitive impairment at that time point likely reflects the speed of lesion spreading to non-motor cortical areas of the brain. Besides, we can assume that King's stages come in succession along the disease course. Indeed, in a recent study using databases from two multicentre clinical trials, 725 patients were retrospectively staged through the trial course. The authors reported that no reversions to earlier disease stages occurred and that most people progressed to the consecutive stage [24]. In ALS longitudinal studies on neuroimaging are challenging, since disability worsens over time: patients may be unable to undergo follow up scans due to severe motor impairment and/or the development of respiratory failure. A recent review [25] evaluated published studies employing neuroimaging to track the course of ALS but did not report any ^{18}F-FDG-PET study.

Another possible limitation should be considered in the interpretation of the asymmetry of the metabolic clusters identified in the analyses, due to the lacking of full information about handedness. Indeed, such data were available only for a part of the patient sample and were unavailable for controls. A further possible limitation is the absence of partial volume effect correction for cortical atrophy. Unfortunately, MRI scans were not available for all subjects. However, studies employing voxel-based atrophy correction of resting glucose metabolism showed that metabolic measurements were relatively independent of brain atrophy [26].

On the other hand, our study increases the knowledge about the neuroimaging correlates of King's stages, which is still limited to few MRI studies with small samples. In this field, a recent study using magnetic resonance (MR) high angular resolution diffusion imaging (HARDI), suggested an early pattern of microstructural degeneration in ALS, mainly involving the CST and the corpus callosum [7]. Nevertheless, in such study patients with early disease stages were not compared to cases with more advanced stages. A further study investigated connectivity alterations associated with the different stages of ALS [8], employing magnetoencephalography and MRI. The authors suggested that modifications, in terms of increased connectedness of functional brain networks, are related to disease progression. These results further support the hypothesis of multi-system involvement of brain networks in ALS. Finally, a longitudinal study [9] reported that the King's ALS disease stages estimated from the ALSFRS-R correlated with CST diffusion measures in *C9orf72* carriers with heterogeneous clinical presentations (asymptomatic subjects, and ALS, ALS-FTD and bvFTD patients). Based on the limited number of mutation carriers in our series, we think that the possible impact of genetic mutations on brain metabolism should be investigated in further studies including a larger amount of carriers.

Another strength of this study is the contribution to the debate about the use of brain ^{18}F-FDG-PET in ALS. Recently, a panel of experts, on behalf of the European Association of Nuclear Medicine

(EANM) and the European Academy of Neurology (EAN), did not recommend the clinical use of brain ^{18}F-FDG-PET even to study ALS-related brain dysfunction [27,28]. We feel that the present data support the use of ^{18}F-FDG-PET to study ALS-related brain changes across motor and cognitive regions in a research setting. Otherwise, in agreement with the EANM-EAN recommendations, its use in the clinical setting needs further studies, including the comparison with ALS-mimic disorders, to achieve a reliable accuracy at single patient level.

To our knowledge, this is the first study evaluating the brain metabolic correlates of regional extent expressed as disease staging in ALS, and includes the largest ALS series with ^{18}F-FDG-PET assessment ever published. We found that, with the increase of King's stage, there was a decrease of metabolism in motor areas, with a progressive involvement of extramotor regions. Since ^{18}F-FDG-PET is a marker of neurodegeneration *in vivo* [10] and is considered valuable in cross-sectionally evaluating the spread of lesions in ALS [29], our data are in keeping with the ALS neuropathological staging model supporting that neurodegeneration extends from the motor cortex to posterior and anterior regions, possibly via axonal pathways [3], and are in agreement with our population-based, cross-sectional data showing that cognitive impairment tends to be more severe as the clinical stage increases [14]. Longitudinal studies are indispensable for the correlation of brain metabolic alterations with the progressive clinical involvement of different body regions and the possible cognitive deterioration over time.

Compliance with Ethical Standards

Conflicts of Interest

Antonio Canosa, Cristina Moglia, Umberto Manera, Rosario Vasta, Francesca Di Pede, Sara Cabras, Davide Nardo, Vincenzo Arena, Maurizio Grassano, Fabrizio D'Ovidio, and Marco Pagani report no disclosures.

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Funding sources had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Protocol approvals and informed consent

The study was approved by the ethical committee "Comitato Etico Interaziendale Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino". The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Patients signed a written informed consent. They did not receive any remuneration for participation.

Data availability statement

Data will be available upon request by interested researchers.

References

1. Montuschi A, Iazzolino B, Calvo A, Moglia C, Lopiano L, Restagno G, et al. Cognitive correlates in amyotrophic lateral sclerosis: a population-based study in Italy. J Neurol Neurosurg Psychiatry. 2015;86:168–73.

2. van Es MA, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, et al. Amyotrophic lateral sclerosis. Lancet Lond Engl. 2017;390:2084–98.

3. Brettschneider J, Del Tredici K, Toledo JB, Robinson JL, Irwin DJ, Grossman M, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol. 2013;74:20–38.

4. Roche JC, Rojas-Garcia R, Scott KM, Scotton W, Ellis CE, Burman R, et al. A proposed staging system for amyotrophic lateral sclerosis. Brain J Neurol. 2012;135:847–52.

5. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Mot Neuron Disord Off Publ World Fed Neurol Res Group Mot Neuron Dis. 2000;1:293–9.

6. Balendra R, Jones A, Jivraj N, Knights C, Ellis CM, Burman R, et al. Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale. Amyotroph Lateral Scler Front Degener. 2014;15:279–84.

7. Trojsi F, Caiazzo G, Di Nardo F, Fratello M, Santangelo G, Siciliano M, et al. High angular resolution diffusion imaging abnormalities in the early stages of amyotrophic lateral sclerosis. J Neurol Sci. 2017;380:215–22.

8. Sorrentino P, Rucco R, Jacini F, Trojsi F, Lardone A, Baselice F, et al. Brain functional networks become more connected as amyotrophic lateral sclerosis progresses: a source level magnetoencephalographic study. NeuroImage Clin. 2018;20:564–71.

9. Floeter MK, Danielian LE, Braun LE, Wu T. Longitudinal diffusion imaging across the C9orf72 clinical spectrum. J Neurol Neurosurg Psychiatry. 2018;89:53–60.

10. Jack CR, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Lowe V, et al. Shapes of the trajectories of 5 major biomarkers of Alzheimer disease. Arch Neurol. 2012;69:856–67.

11. Varrone A, Asenbaum S, Vander Borght T, Booij J, Nobili F, Någren K, et al. EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. Eur J Nucl Med Mol Imaging. 2009;36:2103–10.

12. Della Rosa PA, Cerami C, Gallivanone F, Prestia A, Caroli A, Castiglioni I, et al. A standardized [18F]-FDG-PET template for spatial normalization in statistical parametric mapping of dementia. Neuroinformatics. 2014;12:575–93.

13. Strong MJ, Abrahams S, Goldstein LH, Woolley S, Mclaughlin P, Snowden J, et al. Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. Amyotroph Lateral Scler Front Degener. 2017;18:153–74.

14. Chiò A, Moglia C, Canosa A, Manera U, Vasta R, Brunetti M, et al. Cognitive impairment across ALS clinical stages in a population-based cohort. Neurology. 2019;93:e984–94.

15. Pagani M, Chiò A, Valentini MC, Öberg J, Nobili F, Calvo A, et al. Functional pattern of brain FDG-PET in amyotrophic lateral sclerosis. Neurology. 2014;83:1067–74.

16. Canosa A, Pagani M, Cistaro A, Montuschi A, Iazzolino B, Fania P, et al. 18F-FDG-PET correlates of cognitive impairment in ALS. Neurology. 2016;86:44–9.

17. Henstridge CM, Sideris DI, Carroll E, Rotariu S, Salomonsson S, Tzioras M, et al. Synapse loss in the prefrontal cortex is associated with cognitive decline in amyotrophic lateral sclerosis. Acta Neuropathol (Berl). 2018;135:213–26.

18. Kassubek J, Müller H-P, Del Tredici K, Brettschneider J, Pinkhardt EH, Lulé D, et al. Diffusion tensor imaging analysis of sequential spreading of disease in amyotrophic lateral sclerosis confirms patterns of TDP-43 pathology. Brain J Neurol. 2014;137:1733–40.

19. Müller H-P, Turner MR, Grosskreutz J, Abrahams S, Bede P, Govind V, et al. A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2016;87:570–9.

20. Kassubek J, Müller H-P, Del Tredici K, Lulé D, Gorges M, Braak H, et al. Imaging the pathoanatomy of amyotrophic lateral sclerosis in vivo: targeting a propagation-based biological marker. J Neurol Neurosurg Psychiatry. 2018;89:374–81.

21. Schmidt R, de Reus MA, Scholtens LH, van den Berg LH, van den Heuvel MP. Simulating disease propagation across white matter connectome reveals anatomical substrate for neuropathology staging in amyotrophic lateral sclerosis. NeuroImage. 2016;124:762–9.

22. Meier JM, van der Burgh HK, Nitert AD, Bede P, de Lange SC, Hardiman O, et al. Connectome- Based Propagation Model in Amyotrophic Lateral Sclerosis. Ann Neurol. 2020;87:725–38.

23. van Weehaeghe D, Ceccarini J, Willekens SM, de Vocht J, van Damme P, van Laere K. Is there a glucose metabolic signature of spreading TDP-43 pathology in Amyotrophic Lateral Sclerosis? Q J Nucl Med Mol Imaging Off Publ Ital Assoc Nucl Med AIMN Int Assoc Radiopharmacol IAR Sect Soc Of. 2017;

24. Balendra R, Jones A, Jivraj N, Steen IN, Young CA, Shaw PJ, et al. Use of clinical staging in amyotrophic lateral sclerosis for phase 3 clinical trials. J Neurol Neurosurg Psychiatry. 2015;86:45–9.

25. Chipika RH, Finegan E, Li Hi Shing S, Hardiman O, Bede P. Tracking a Fast-Moving Disease: Longitudinal Markers, Monitoring, and Clinical Trial Endpoints in ALS. Front Neurol. 2019;10:229.

26. Ibáñez V, Pietrini P, Alexander GE, Furey ML, Teichberg D, Rajapakse JC, et al. Regional glucose metabolic abnormalities are not the result of atrophy in Alzheimer's disease. Neurology. 1998;50:1585–93.

27. Nobili F, Arbizu J, Bouwman F, Drzezga A, Agosta F, Nestor P, et al. European Association of Nuclear Medicine and European Academy of Neurology recommendations for the use of brain 18 F-fluorodeoxyglucose positron emission tomography in neurodegenerative cognitive impairment and dementia: Delphi consensus. Eur J Neurol. 2018;25:1201–17.

28. Agosta F, Altomare D, Festari C, Orini S, Gandolfo F, Boccardi M, et al. Clinical utility of FDG-PET in amyotrophic lateral sclerosis and Huntington's disease. Eur J Nucl Med Mol Imaging. 2018;45:1546–56.

29. Kassubek J, Pagani M. Imaging in amyotrophic lateral sclerosis: MRI and PET. Curr Opin Neurol. 2019;32:740–6.

Figure Captions

Figure 1

Glass brain rendering of multiple regression of King's stage against whole brain metabolism in the whole sample. The clusters showing a statistically significant negative correlation are projected on brain surface.

Figure 2

Glass brain rendering of the comparison: King's stage 1+2 group *versus* King's stage 3 group. The clusters showing a statistically significant relative hypometabolism in the King's stage 3 group as compared to the King's stage 1+2 group are projected on brain surface.

Supplemental Figure 1

Glass brain rendering of the full factorial analysis including the following groups: King's stage 1, King's stage, 2, King's stage 3, and healthy controls. The clusters showing a significant main effect of groups are projected on brain surface.

Supplemental Figure 2

Glass brain rendering of the comparison: King's stage 1 *versus* healthy controls. The clusters showing a statistically significant relative hypometabolism in the King's stage 1 group as compared to healthy controls are projected on brain surface.

Supplemental Figure 3

Glass brain rendering of the comparison: King's stage 2 *versus* healthy controls. The clusters showing a statistically significant relative hypometabolism in the King's stage 2 group as compared to healthy controls are projected on brain surface.

Supplemental Figure 4

Glass brain rendering of the comparison: King's stage 3 *versus* healthy controls. The clusters showing a statistically significant relative hypometabolism in the King's stage 3 group as compared to healthy controls are projected on brain surface.

Table 1. Demographic and clinical characteristics of patients belonging to the three groups (King's Stage 1, 2, and 3) and of the whole sample. Data about the presence/absence of genetic mutations were available for 369 out of 390 patients. The neuropsychological assessment was available for 267 out of 390 patients: cognitive status was classified according to diagnostic criteria published by Strong et al. [13]

		King's Stage			P value	Total
		1 (n=165)	2 (n=133)	3 (n=92)		(n=390)
Sex	F (%)	69 (41.8%)	58 (43.6%)	52 (56.5%)		179 (45.9%)
	M (%)	96 (58.2%)	75 (56.4%)	40 (43.5%)	p=0.062	211 (54.1%)
	Total	165	133	92		390
Age at PET Median (IQR)		64.2 (57.5-70.9)	64.0 (53.6-72.6)	67.1 (59.9-73.7)	p=0.076	64.6 (56.6-71.9)
Age at diagnosis Median (IQR)		63.9 (57.9-70.3)	63.2 (53.2-72.0)	66.8 (59.5-73.4)	p=0.096	64.42 (56.4-71.7)
Disease duration (months) at PET Median (IQR)		11.7 (7.9-16.9)	11.9 (9.2-16.5)	13.2 (8.7-20.6)	p=0.449	12.1 (8.5-17.9)
Site of Onset	Bulbar (%)	58 (35.2%)	28 (21.1%)	41 (44.6%)		127 (32.6%)
	Spinal (%)	107 (64.8%)	105 (78.9%)	51 (55.4%)	p<0.001	263 (67.4%)
	Total	165	133	92		390
***C9orf72* repeat expansion**	Negative (%)	146 (90.1%)	108 (89.3%)	81 (94.2%)	p=0.447	335 (90.8%)

	Positive (%)	16 (9.9%)	13 (10.7%)	5 (5.8%)		34 (9.2%)
	Total	162	121	86		369
***SOD1* mutations**	Negative (%)	157 (96.9%)	119 (98.3%)	84 (97.7%)		360 (97.6%)
	Positive (%)	5 (3.1%)	2 (1.7%)	2 (2.3%)	p=0.750	9 (2.4%)
	Total	162	121	86		369
***TARDBP* mutations**	Negative (%)	157 (96.9%)	117 (96.7%)	83 (96.5%)		357 (96.7%)
	Positive (%)	5 (3.1%)	4 (3.3%)	3 (3.5%)	p=0.986	12 (3.3%)
	Total	162	121	86		369
***FUS* mutations**	Negative (%)	161 (99.4%)	118 (97.5%)	86 (100%)		365 (98.9%)
	Positive (%)	1 (0.6%)	3 (2.5%)	0 (0%)	p=0.185	4 (1.1%)
	Total	162	121	86		369
ALS FRS-R Total Score Median (IQR)		45 (43-46)	39 (37-42)	33.50 (30-37)	p<0.001	41 (36-44)
Cognitive Status	ALS-Cn (%)	70 (58.3%)	38 (44.7%)	26 (41.9%)		134 (50.2%)
	ALS-bi (%)	21 (17.5%)	22 (25.9%)	16 (25.8%)		59 (22.1%)
	ALS-ci (%)	16 (13.3%)	13 (15.3%)	3 (4.8%)	p=0.019	32 (12.0%)
	ALS-cbi (%)	7 (5.8%)	8 (9.4%)	7 (11.3%)		22 (8.2%)
	ALS-FTD (%)	6 (5.0%)	4 (4.7%)	10 (16.1%)		20 (7.5%)
	Tot	120	85	62		267

F: female. M: male. IQR: interquartile range. ALSFRS-R: ALS Functional Rating Scale – Revised. ALS-Cn: ALS with normal cognition. ALS-bi: ALS with behavioural impairment. ALS-ci: ALS with cognitive impairment. ALS-cbi: ALS with cognitive and behavioural impairment. ALS-FTD: ALS with frontotemporal dementia.

Table 2. Results of the negative correlation between whole brain metabolism and King's stage in the whole sample (BA=Brodmann Area).

P (FWE-corr)	Cluster Extent	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
0.001	1298	4.91	-10	-24	71	Frontal	Left Medial Frontal Gyrus	6
		3.68	12	-11	56	Frontal	Right Medial Frontal Gyrus	6
0.021	658	4.05	44	-15	50	Frontal	Right Precentral Gyrus	4
		3.98	44	-23	47	Parietal	Right Postcentral Gyrus	2

Table 3. Clusters showing a statistically significant relative hypometabolism in the King's stage 3 group as compared to the King's stage 1+2 group (BA=Brodmann Area).

p(FWE-corr)	Cluster Extent	Z-score	Talairach Coordinates			Lobe	Cortical Region	BA
0.000	3321	5.11	-42	-9	56	Frontal	Left Precentral Gyrus	4
		4.67	-8	-18	64	Frontal	Left Precentral Gyrus	6
		4.41	-10	-24	71	Frontal	Left Medial Frontal Gyrus	6
0.000	2089	4.46	38	-13	54	Frontal	Right Precentral Gyrus	4
		4.40	40	12	42	Frontal	Right Middle Frontal Gyrus	6
		4.05	44	-21	40	Parietal	Right Postcentral Gyrus	3
0.006	899	3.58	8	40	24	Frontal	Right Medial Frontal Gyrus	9
		3.45	6	29	41	Frontal	Right Medial Frontal Gyrus	8

Figure 1

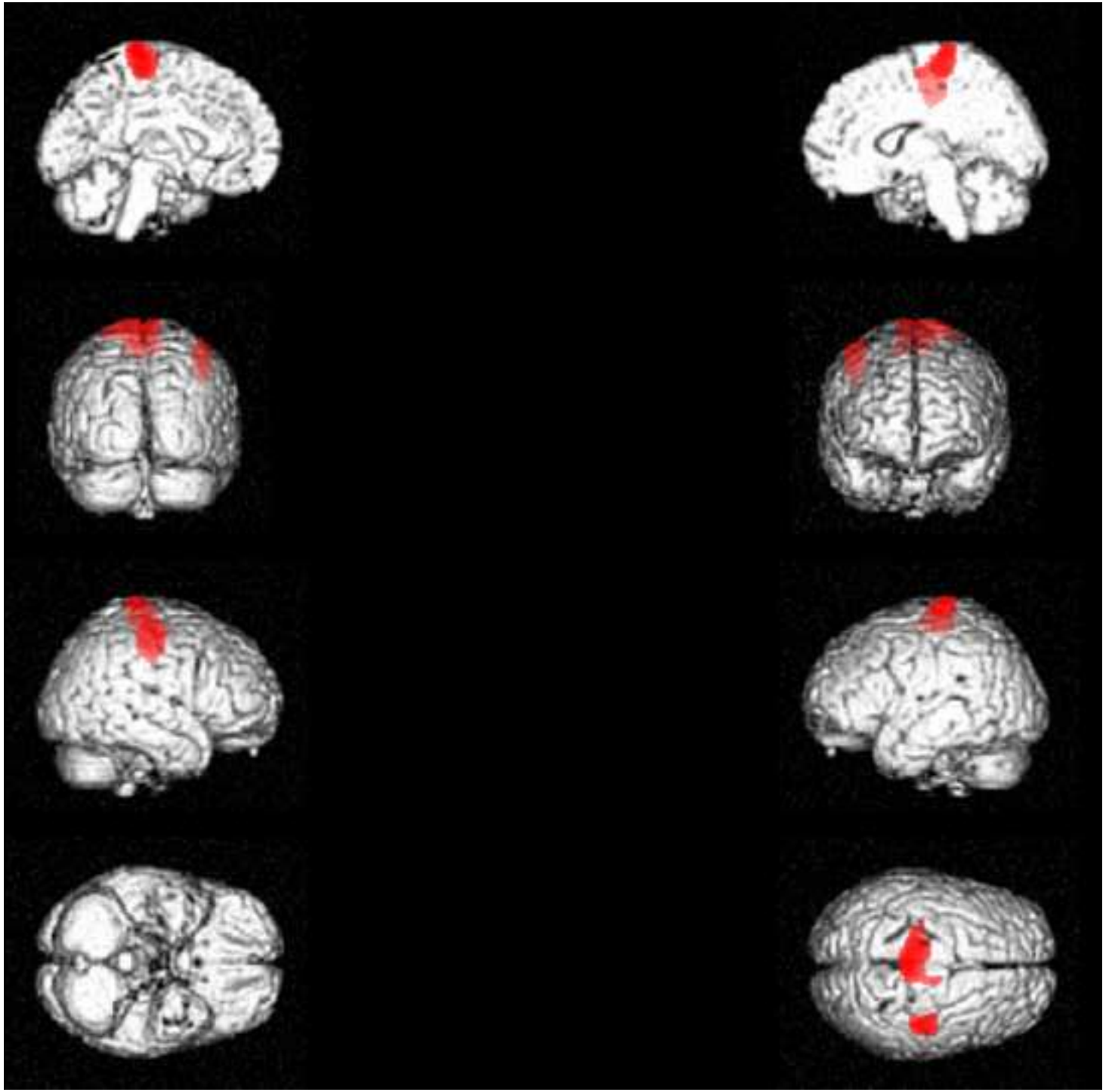
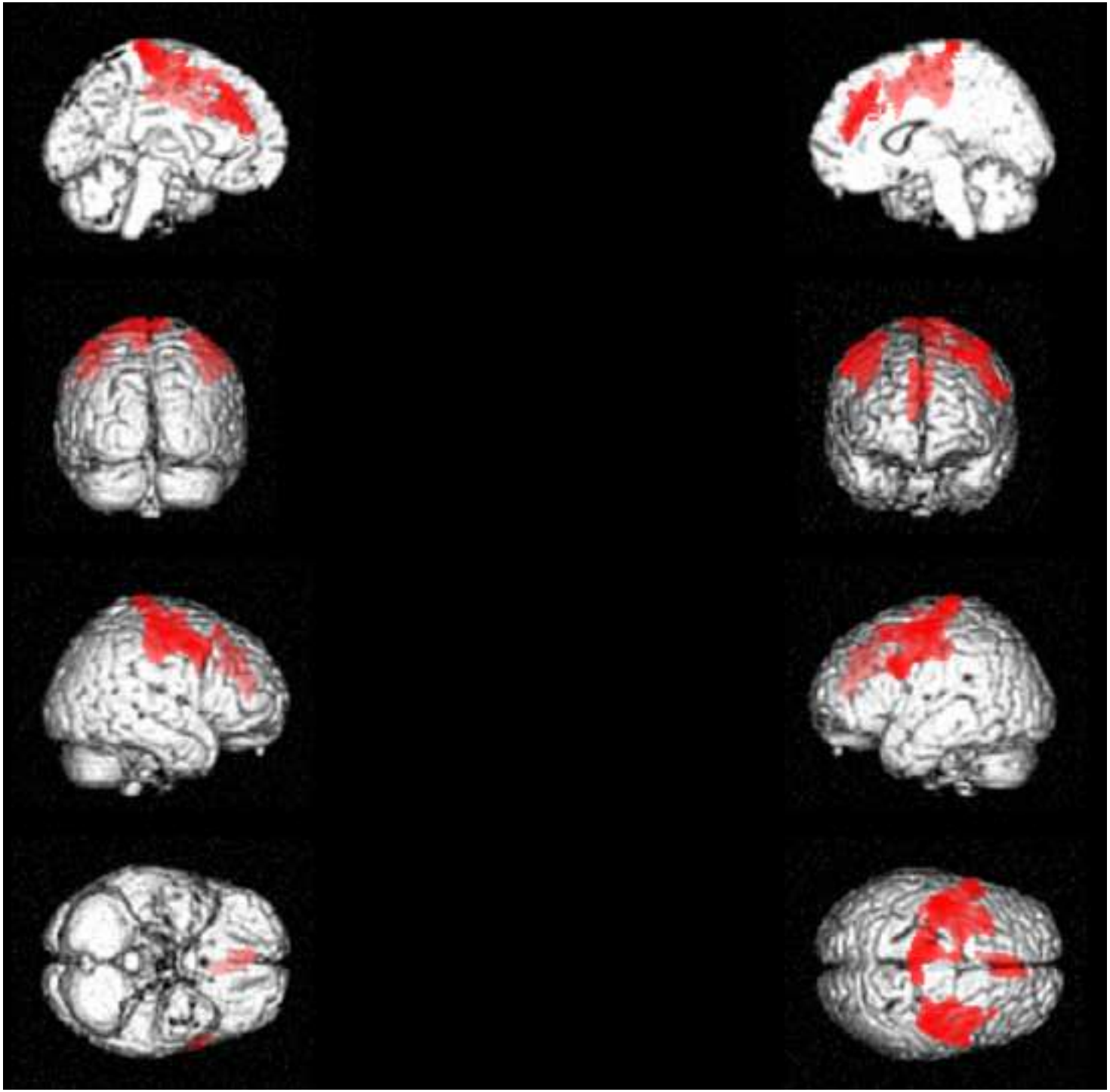


Figure 2



Supplementary Figure 1

Supplementary Figure 2

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